dependent imaging protocol as previously described. The tumor is insonified to induce localized hyperthermia. The temperature of targeted tissue is monitored continuously by the changes in acoustic backscatter. Incremental temperature differences are color-mapped onto ultrasonic image displays that are repetitively updated and reviewed by the operator. The operator manually or the equipment automatically adjusts intensity or frequency of the ultrasonic beam to optimize tumor destruction and minimize collateral damage to normal tissues.

A7

AMENDED CLAIMS REWRITTEN IN CLEAN FORM:

18. (Amended) A method for measuring enhanced acoustic reflectivity of an ultrasound target, the method comprising (1) administering to the target, a nongaseous acoustic imaging substance which binds to the target and produces a change in acoustic reflectivity with a change in temperature and (2) changing the temperature to produce a measurable change in acoustic reflectivity of the nongaseous acoustic imaging substance bound to the target, and (3) detecting change in acoustic reflectivity of the bound substance.

A8 Sub R6

63. (Amended) The device according to claim 54 wherein the component configured to change the temperature of the acoustic imaging substance is configured to change the temperature of the bound substance by at least 5°C.

A9

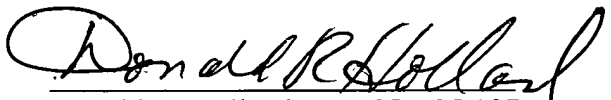
Remarks

This paper amends the specification and Claims 18 and 63 to correct typographical and clerical errors. The change in Claim 18 constitutes correction of a clerical error and the change in Claim 63 constitutes correction of a typographical error. With respect to the change in Claim 18, it is noted that dependent Claim 31 is directed to energizing to increase temperature of the bound substance and dependent Claim 33 is directed to reducing temperature of the bound substance so that Claim 18 includes changing temperature by increasing temperature and changing temperature by decreasing temperature. This amendment does not narrow the scope of the claims and it does not

constitute an amendment for any reason related to the statutory requirements for a patent. Furthermore, no new matter is believed to be added in the amendments to the specification and claims. Claims 1-67 remain pending in the case.

It is requested that the amendments above be entered and that the claims be examined on the merits. Should any questions arise, the Patent and Trademark Office is requested to contact the undersigned attorney.

Respectfully submitted,



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SPECIFICATION REPLACEMENT PARAGRAPHS IN MARKED-UP FORM

On page 1, line 8 through page 2, line 2, please delete paragraph and substitute therefor, the paragraph below shown in marked-up version which has been changed with respect to the original paragraph by deleting on line 25, the letter "m" after the numeral "1998".

Molecular imaging can enhance the utility of traditional clinical imaging by allowing specific detection of molecular markers in tissues using site-targeted contrast agents (Weissleder, *Radiology 212*:609-614, 1999). Three approaches to site-targeted ultrasonic agents have been reported and these are based upon the use of liposomes (Alkan-Onyuksel et al., *J. Pharm. Sci 85*:486-490, 1996; Demos et al., *J. Pharm. Sci. 86*:167-171, 1997; Demos et al., *J. Am. Col. Cardiol. 33*:867-875, 1999), the use of microbubbles (Mattrey et al, *Am. J. Cardiol. 54*:206-210, 1984; Unger et al., *Am. J. Cardiol. 81*:58G-61G, 1998; Villanueva et al, *Circulation 98*:1-5, 1998; Klibanov et al, *Acad. Radiol. 5*S243-S246, 1998) or the use of nano-emulsions (Lanza et al, *Circulation 94*:3334-3340, 1996; Lanza et al, *J. Acoust. Soc. Am. 104*:3665-3672, 1998; Lanza et al, *Ultrasound Med. Biol. 23*: 863-870, 1997). Liposomes are spherical bimembrane vesicles produced spontaneously by phospholipids in water. Multilamellar lipid bilayers produced through a dehydration-rehydration process can form internal vesicles within a liposome and lead to increased acoustic reflectance (Alkan-Onyuksel et al., 1996 *supra*; Demos et al., 1997, *supra*; Demos et al., 1999, *supra*). In the second approach, microbubbles have been proposed for site-targeted modalities in addition to their perfusion applications. Microbubbles have been targeted towards thrombi (Unger et al., 1998 **[m]** supra; Lanza et al., *Ultrasound. Med. Biol. 23*: 863-870, 1997), avidin-coated petri dish (Klibanov et al, 1998, *supra*) and activated endothelial cells (Villanueva et al, 1998, *supra*). Other investigators have examined the interaction of thrombus with site targeted agents. In particular, Unger et al. has observed successful binding of MRX-408, a bubble-based contrast agent, both *in vitro* and *in vivo* (Unger et al., 1998, *supra*).

On page 4, lines 12-24, please delete paragraph and substitute therefor, the paragraph below shown in marked-up version, which has been changed with respect to the original paragraph by deleting on line 18, the words "at increased temperature" after the words "bound substance".

In another embodiment, the present invention comprises a method for measuring enhanced acoustic reflectivity of an ultrasound target. The method comprises (1) administering to the target, a nongaseous acoustic imaging substance which binds to the target and produces a change in acoustic reflectivity with a change in temperature and (2) changing the temperature to produce a measurable change in acoustic reflectivity of the nongaseous acoustic imaging substance bound to the target, and (3) detecting change in acoustic reflectivity of the bound substance **[at increased temperature]**. Detecting the change in acoustic reflectivity, preferably, comprises (a) measuring reflectivity prior to changing the temperature of the bound substance; (b) measuring reflectivity after changing the temperature of the bound substance; and (c) determining the change in reflectivity after changing the temperature of the bound substance compared to reflectivity prior to changing the temperature of the bound substance.

On page 7, line 29 through page 8, line 16, please delete paragraph and substitute therefor, the paragraph below shown in marked-up version, which has been changed with respect to the original paragraph by adding on page 8, line 10, the word "of" after the word "emulsions".

The oil phase of the oil-in-water emulsion comprises, preferably, 5 to 50% and, more preferably 20 to 40% by weight of the emulsion. The oil or hydrophobic constituent exhibits an acoustic impedance that varies with changes (i.e. positively or negatively) in temperature, preferably, a fluorochemical liquid. These include straight, branched chain and cyclic perfluorocarbons, straight , branched chain and cyclic perfluoro tertiary amines, straight , branched chain and cyclic perfluoro ethers and thioethers, chlorofluorocarbons and polymeric perfluoro ethers and the like. Although up to 50% hydrogen-substituted compounds can be used, perhalo compounds are preferred.

Most preferred are perfluorinated compounds. Any fluorochemical liquid, i.e. a substance which is a liquid at or above body temperature (e.g. 37°C) at atmospheric pressure, can be used to prepare a fluorochemical emulsion of the present invention. However, for many purposes emulsions **of** fluorochemicals with longer extended stability are preferred. In order to obtain such emulsions, fluorochemical liquids with boiling points above 50°C are preferred, and most preferred are fluorochemical liquids with boiling points above about 80°C. The guiding determinant should be that the oil, e.g. a fluorochemical, should be expected to remain in a liquid phase (less than10% gas conversion) under the intended conditions of thermal induction and imaging.

On page 32, lines 1-7, please delete paragraph and substitute therefor, paragraph below shown in marked-up version, which has been changed with respect to the original paragraph by deleting on line 5, the word "is" and substituting therefor the word "are" after the word "fragments".

F(ab) fragments are generated and isolated using an immunopure F(ab) preparation kit (Pierce, Rockford, IL). Briefly, IgG is dialyzed into 20mM phosphate/10mM EDTA buffer (pH 7.0), concentrated to 20 mg/ml and digested by immobilized papain. Solubilized F(ab) is purified from Fc fragments and undigested IgG protein using a protein A column. F(ab) fragments **[is] are** purified from excess cysteine using a G25-150 column and deoxygenated phosphate buffer (pH 6.7). Fraction identity is confirmed by routine SDS-PAGE procedures.

On page 34, lines 22-28, please delete paragraph and substitute therefor, the paragraph below shown in marked up version, which has been changed with respect to the original paragraph by deleting on line 25, the misspelling "Dehyrdated" and substituting therefor the word "Dehydrated".

Avidin (50 μg) dissolved in 0.1 M phosphate buffered saline (PBS) (pH 7.2-7.4) was spotted and air-dried dropwise onto the center of each membrane with a microliter syringe and allowed to dry. Next, each membrane was washed with three, five-minute

changes of PBS-0.1% Tween 20. **[Dehyrdated]** **Dehydrated** milk powder suspended in PBS-0.1% Tween 20 was used to block glutaraldehyde activated protein binding sites left unoccupied after the application of avidin for 20 minutes. Excess protein was removed with three, five minute isotonic, PBS washes.

On page 38, line 17 through page 39, line 3, please delete paragraph and substitute therefor, the paragraph below shown in marked-up version, which has been changed with respect to the original paragraph by deleting on page 39, line 1, the misspelling "hyperthermetry" before the word "protocol" and substituting therefor the word "hyperthermometry".

The keys to all this regimen, especially with regard to difficult to distinguish small tumors, are (1) the precise localization and morphologic delineation of the tumor in a three-dimensional volume space and (2) noninvasive thermometry of the tissue heating process to ensure tumor kill and sparing of normal collateral tissues.

The temperature-dependent ultrasound contrast agents of the present invention will greatly enhance the high-resolution detection, localization and mapping of tumors in two-dimensional or three-dimensional space, particularly when the cancer is small or the background in inherently acoustically reflective. This is achieved through the differential ultrasonic response of nanoparticle-targeted and surrounding normal tissues. In addition, the temperature-dependent changes in acoustic backscatter could be used an internal thermometry, assuring that targeted tissues are heated to appropriate levels while of other tissue heating is minimized. This high resolution, noninvasive thermometry may be constantly displayed in real-time using a scaled color map to allow the operator to monitor tissue temperatures and manually adjust the hyperthermetry protocol. Alternatively, ultrasonic beam adjustments may be automatically implemented "on-the-fly" by the **[hyperthermia]** **hyperthermometry** machine through self-monitoring algorithms.

On page 41, lines 3-19, please delete paragraph and substitute therefor, the paragraph below shown in marked-up version, which has been changed with respect to

the original paragraph by deleting on line 17, the word "repetitive" before the word "updated" and substituting therefor the word "repetitively" and by deleting on line 19 after the word "optimize" the misspelling "tomor" and substituting therefor the word "tumor".

The catheter for hyperthermia and ultrasound system could be used in treating cancer as in the following example. A patient with suspected pancreatic cancer, would be admitted to the hospital, and an intravenous catheter placed. Temperature-sensitive acoustic nanoparticles bearing a monoclonal antibody fragment directed against $\alpha_v\beta_3$ integrin on neovascular cells is administered at a dose of between 0.1 and 1.0 ml/kg body weight, preferably 0.25 to 0.5 ml/kg. The agent is allowed to circulate and saturate the neovascular tissue receptors for between 15 minutes and 5 hours, preferably 1 to 2 hours. A combinational therapeutic/imaging ultrasonic catheter is advanced and images of the pancreas from a transgastric/transduodenal approach are obtained. The diagnosis, location and extend of the pancreatic tumor is confirmed through a temperature-dependent imaging protocol as previously described. The tumor is insonified to induce localized hyperthermia. The temperature of targeted tissue is monitored continuously by the changes in acoustic backscatter. Incremental temperature differences are color-mapped onto ultrasonic image displays that are **[repetitive]** **repetitively** updated and reviewed by the operator. The operator manually or the equipment automatically adjusts intensity or frequency of the ultrasonic beam to optimize **[tomor]** **tumor** destruction and minimize collateral damage to normal tissues.

AMENDED CLAIMS IN MARKED UP FORM

18. (Amended) A method for measuring enhanced acoustic reflectivity of an ultrasound target, the method comprising (1) administering to the target, a nongaseous acoustic imaging substance which binds to the target and produces a change in acoustic reflectivity with a change in temperature and (2) changing the temperature to produce a measurable change in acoustic reflectivity of the nongaseous acoustic imaging substance bound to the target, and (3) detecting change in acoustic reflectivity of the bound substance , [at increased]

63. (Amended) The device according to claim 54 wherein the component [confugured] configured to change the temperature of the acoustic imaging substance is configured to change the temperature of the bound substance by at least 5°C.